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# Use of response surface methodology to study the combined effects of UV-C and thermal processing on vegetable oxidative enzymes☆

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ABSTRACT

The combined thermal (25–65 °C) and ultraviolet processing (UV-C) effects on lipoxygenase (LOX), peroxidase (POD) and polyphenoloxidase (PPO) at different pH values (4.0–7.0) were studied using a central composite design. An initial screening design revealed that all factors had a significant effect on enzymatic activity except wavelength which showed a negligible effect. A synergistic effect was found between temperature and UV exposure time for POD and PPO and between pH and exposure time for LOX. LOX enzyme was affected by acidic conditions. POD was UV-C labile whereas PPO was the most UV-C resistant enzyme but was thermolabile. Second-order polynomial equations indicated that enzyme activities were inactivated after exposure to 58.2 mJ/cm$^2$ UV at 60 °C or higher temperatures at any pH condition. Combination of UV and thermal processing allowed the use of low energy/doses to obtain complete enzymatic inactivation. This study may serve as a basis to design UV-C processes for the inactivation of enzymes in liquid matrices.

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## 1. Introduction

Consumer tendencies are moving toward an increasing demand for more convenient, nutritious, fresh and reasonably priced products. Traditional technologies such as thermal processing are in some cases unable to meet those requirements due to undesirable changes occurring in the product compromising the nutritional and sensory quality. For those products, market niches have appeared for emerging technologies to preserve their fresh-like character and nutritional quality (Sampedro, Fan, & Rodrigo, 2010).

Ultraviolet light (UV-C) technology has been used for decades as a well-established technology for water treatment, air disinfection and surface decontamination; however, it is still considered as an emerging technology for food processing (Koutchma, 2009). UV-C processing has shown promise in the reduction of foodborne pathogens in different liquid food matrices (Koutchma, Forney, & Moraru, 2009, chap. 1). The theory underlying UV germicidal effects is that the damage done to nucleic acids disrupts the DNA and RNA structures of bacteria thereby preventing them from replicating (Guerrero-Beltran & Barbosa-Cánovas, 2004). The successful microbial inactivation results together with reduced operating and maintenance costs obtained with new UV equipment have led the industry to obtain the U.S. Food and Drug Administration (FDA) premarket approval (21 CFR 179.39) for treatment of water and foods with UV-C technology (FDA, 2012).

Quality-related enzymes play an important role when designing a new treatment for the preservation of vegetable-based food products such that a certain degree of enzyme inactivation must be achieved to avoid detrimental quality effects (Van Loey, Indrawati, & Hendrickx, 2003). In conventional thermal processes applied to acidic foods and for pasteurization of dairy products, enzymes are often used as indicators partially because heat resistance of many enzymes is greater than that of pathogenic microorganisms (Basak & Ramaswamy, 1996).

Enzymes such as polyphenoloxidase (PPO), peroxidase (POD) and lipoxygenase (LOX) govern degradative processes that occur in fruits and vegetables (Ludikhuyze, Van Loey, Indrawati, Smout, & Hendrickx, 2003). PPO is mainly responsible for the oxidation of phenolic compounds that leads to enzymatic browning. LOX

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comprises a group of enzymes that catalyze lipid oxidation and pigment bleaching, thereby inducing changes in flavor, color and nutritional value. POD plays a role in the development of off-flavors and discoloration in raw and unblanched vegetables (Ludikhuyze, Van Loey, Indrawati, & Hendrickx, 2002). Resistance of enzymes to food processing technologies depends on several factors such as their three-dimensional structure, the enzyme source, food matrix composition and processing conditions (Sampedro et al., 2010).

Several authors have studied the effects of UV-C processing on enzymatic activity of POD, PPO and LOX in whole fruits (Chisari, Barbagallo, Spagna, & Artes, 2011; Gonzalez-Aguilar, Zavaleta-Gatica, & Tiznado-Hernandez, 2007), fruit products (Falguera, Pagan, & Ibarz, 2011; Guerrero-Beltran & Barbosa-Cánovas, 2006) and combined UV-C and pulsed electric fields (Noci et al., 2008). The researchers showed enzymes were inactivated in various degrees ranging from complete inactivation to no effect by using UV exposure times from 15 to 100 min. Despite the different studies found in the scientific literature, there are no studies up to date about the enzyme behavior after a combined UV-C and thermal processing. A more complete experimental design including processing parameters such as temperature, UV exposure time and wavelength, and environmental factors such as pH will allow for a better understanding of enzyme behavior after UV-C processing.

Therefore, the objective of this study is to characterize the response of different quality-deteriorative oxidative enzymes to a combined thermal and UV-C treatment.

## 2. Material and methods

### 2.1. Enzyme samples

Lipoxygenase (LOX) from soybean (EC 1.13.11.12) containing 158,000 units/mg solid, peroxidase (POD) from horseradish (EC 1.11.1.7) containing 256 units/mg solid and polyphenoloxidase (PPO) from mushroom (EC 1.14.18.1) containing 98,800 units/mg solid were purchased as dry powders (Sigma–Aldrich, St. Louis, MO). Enzymes were dissolved in buffer solutions at different pH values within the range of 4.0–7.0 corresponding to those naturally found in vegetable and fruit matrices (juices and beverages). Sodium-phosphate buffer (0.2 mol/L, pH 7.0, 6.25, 5.5) and sodium-acetate buffer (0.2 mol/L, pH 4.0, 4.75). Enzymes were added at concentrations close to those naturally found in liquid vegetable and fruit matrices as 50 μg/mL (LOX) in tomato juice (Rodrigo, Jolie, Van Loey, & Hendrickx, 2007), 20 μg/mL (POD) in orange juice (Cano, Hernandez, & De Ancos, 1997) and 35 μg/mL (PPO) in mushroom (Weemaes et al., 1997).

### 2.2. Enzyme assay

Stability of dissolved enzymes at refrigeration conditions was studied during the time for processing and analysis, confirming that no loss of activity was produced (data not shown). Calibration curves were calculated with different buffers used at decreasing concentrations to check the linearity of the measurement and obtain the detection limit (data not shown). All the chemicals used for the enzymatic analysis were obtained from Sigma–Aldrich (St. Louis, MO).

#### 2.2.1. LOX activity

LOX activity was measured according to a method described by Rodrigo et al. (2007) and Aguiló-Aguayo, Sobrino-López, Soliva-Fortuny, and Martín-Belloso (2008) with slight modifications. LOX activity measurement was based on the formation of conjugated dienes from linoleic acid, using sodium linoleate as the substrate. The substrate was prepared by adding 10 μL of linoleic acid and 5 μL of Tween-20 to 4 mL of $H_2O$ followed by 2 min shaking, solubilized with 1 mL of 0.1 N NaOH and diluted to 25 mL with water. The enzyme measurement was carried out by adding 800 μL of enzyme and 400 μL of substrate to 2.7 mL of sodium-phosphate buffer (0.2 mol/L, pH 6.5) to a quartz cuvette at 25 °C. The absorption at 234 nm using a spectrophotometer (Shimadzu UV-2401PC, Shimadzu Scientific Instruments Inc. Columbia, MD) was recorded every second for 300 s and activity was determined from the slope of the linear portion of the curve. One unit of enzyme is defined as the amount of enzyme producing one unit change in absorbance per minute at 25 °C.

#### 2.2.2. POD activity

POD assay was based on a procedure described by (Cano et al., 1997; Garcia-Palazon, Suthanthangjai, Kajda, & Zabetakis, 2004) with slight modifications. Ten microliters of the enzyme were mixed with 0.2 mL of *p*-phenylenediamine (1 g/mL), 0.1 mL hydrogen peroxide (1.5 mL/100 mL) and 2.7 mL sodium-phosphate buffer (0.05 mol/L, pH 6.5) in a quartz cuvette at 25 °C. Absorbance was recorded at 485 nm every second for 90 s. The slope of the linear part of the curve (first 60 s) was determined and one unit of enzyme was defined as the change in absorbance per minute at 25 °C.

#### 2.2.3. PPO activity

PPO activity was based on the procedure described by Weemaes et al. (1997) with some modifications. Two hundred microliters of the enzyme were added to 1 mL of catechol solution (0.01 mol/L in phosphate buffer, pH 6.5, 0.1 mol/L) in a quartz cuvette at 25 °C. The change of absorbance was recorded at 411 nm for 90 s and the slope of the linear part of the curve (first 30 s) was determined. One unit of enzyme was expressed as the change of absorbance per minute at 25 °C.

### 2.3. UV-C treatment

Fig. 1 shows the UV-C system design. The UV treatment was conducted in a stainless steel rectangular cabinet (68.5 × 44 × 28 cm) with openings on both sides (8 × 44 cm). Two ultraviolet low-pressure irradiator bulbs (61 cm in length) with maximum emissions of selectable 185 and 254 nm were mounted 18 cm apart in germicidal lamp fixtures inside the cabinet 2.5 cm from the center, and 19 cm from the bottom (Atlantic UltraViolet Corp., Hauppauge, NY). Fifteen mL of enzyme sample was distributed as a thin layer (<5 mm) in a glass dish (100 × 15 mm) and placed on a stirring plate with a flexible silicone rubber heater attached to it (Omega Engineering Inc., Stamford, CT). A PID controller (Model CN4431, Omega Engineering Inc.) was used to maintain the temperature constant during the process by supplying

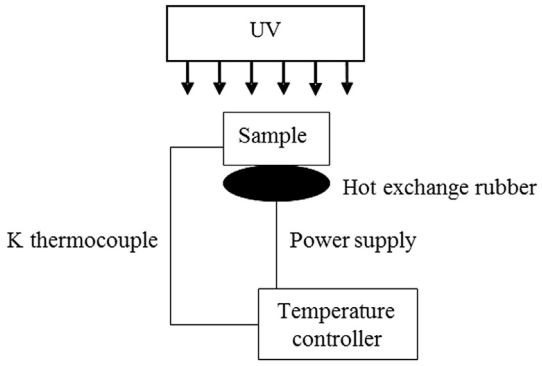


**Fig. 1.** Schematic of UV-C treatment system.

energy to the heater. A thermocouple (K type) in contact with the sample was used to monitor temperature. The sample was located at 7.5 cm from the UV source. A radiometer (UV-340, Lutron Electronic Ent. Co., Ltd. Taipei, Taiwan) was used to measure the intensity flux expressed as $\mu W/cm^2$. Samples were irradiated for different exposure times and collected in glass tubes, wrapped with aluminum foil to avoid photoreactivation and immediately immersed in an ice-water bath until the analysis of enzyme activity. The different doses of UV-C radiation were calculated by using the following equation (Guerrero-Beltran & Barbosa-Cánovas, 2004; Stevens et al., 1999):

$$D = I_{254} \times t \tag{1}$$

where $D$ is the dose ($mJ/cm^2$), $I_{254}$ is the intensity flux ($\mu W/cm^2$) and $t$ is the exposure time (s).

Enzyme activity of control (untreated) and UV-treated samples were measured and residual activity $A_{res}$ was expressed as:

$$A_{res} = \frac{A}{A_0} \tag{2}$$

where $A$ is the enzyme activity after the treatment and $A_0$ is the initial activity (untreated). All determinations were carried out in triplicate.

### 2.4. Experimental design

#### 2.4.1. Screening design

A screening design was first applied to estimate the magnitudes of the effect of each processing factor on enzyme activity to select the main influential factors. UV-C processing factors (exposure time and wavelength), temperature, and pH were selected by choosing low and high levels of each factor that mimic the range of operational conditions applicable to the food industry. For temperature, 25 and 65 °C were selected as the minimum and maximum values for the common range used in nonthermal processing conditions, treatment time was selected from 1 to 10 min as short and long treatment times, pH from 4.0 to 7.0 to study the range of pH values naturally found in vegetable and fruit matrices and 185 and 254 nm were selected for wavelength values within the germicidal range (Table 1). The effect of each factor on enzyme activity was estimated by the following equations (each run done in triplicate):

Effect of temperature

$$0.25*[(\text{Run2} + \text{Run4} + \text{Run6} + \text{Run8}) - (\text{Run1} + \text{Run3} + \text{Run5} + \text{Run7})] \tag{3}$$

Effect of time

$$0.25*[(\text{Run3} + \text{Run4} + \text{Run7} + \text{Run8}) - (\text{Run1} + \text{Run2} + \text{Run5} + \text{Run6})] \tag{4}$$

Effect of pH

$$0.25*[(\text{Run5} + \text{Run6} + \text{Run7} + \text{Run8}) - (\text{Run1} + \text{Run2} + \text{Run3} + \text{Run4})] \tag{5}$$

Effect of wavelength

$$0.25*[(\text{Run2} + \text{Run3} + \text{Run5} + \text{Run8}) - (\text{Run1} + \text{Run4} + \text{Run6} + \text{Run7})] \tag{6}$$

where run $i$ denotes the enzyme activity observed for the $i$th run as shown in Table 1.

Negative values indicated that high factor settings produced lower enzyme activity (higher inactivation) whereas positive values indicated that lower settings produced lower activity.

**Table 1**
Screening design for the effects of four factors (temperature, treatment time, pH and UV-C wavelength) on enzyme activities. Each factor had two levels.

| Run | Factors | | | |
|---|---|---|---|---|
| | Temperature | Time | pH | Wavelength |
| 1 | L | L | L | L |
| 2 | H | L | L | H |
| 3 | L | H | L | H |
| 4 | H | H | L | L |
| 5 | L | L | H | H |
| 6 | H | L | H | L |
| 7 | L | H | H | L |
| 8 | H | H | H | H |

H: high level.
L: low level.

#### 2.4.2. Central composite design

The information from the screening design was used to select the three most significant factors for each enzyme. A central composite design (CCD) for three quantitative factors at five levels (coded as −2, −1, 0, +1, +2) was used to study the response on the residual enzyme activity. A total of 20 runs (done in triplicate) (14 combinations plus 6 center points) guaranteed only minor departures from rotatability and orthogonality for the experimental design. Experimental data were fitted to the following second-order polynomial model:

$$A_{res} = \beta_0 + \sum_i \beta_i x_i + \sum_i \beta_{ii} x_i^2 + \sum_{i,j} \beta_{ij} x_i x_j \tag{7}$$

where $A_{res}$ is the response variable as the residual enzyme activity after the treatment, $\beta_0$, $\beta_i$, $\beta_{ii}$ and $\beta_{ij}$ are regression coefficients for intercept, linear, quadratic and interaction, respectively. The goodness of the fit for the developed regression models was tested by $F$ value, $R^2$ and mean square error (MSE). Contour plots of the response surfaces were obtained using the fitted model by keeping the least effective independent variable at a constant while changing the other two variables. The regression analysis and contour graphs were obtained by using SAS® software (Version 9.3, SAS Institute, Cary, NC).

## 3. Results and discussion

### 3.1. Screening of UV-C processing factors

The UV-C screening experiments were carried out in the design as shown in Table 1. The UV setting allowed an accurate control of the process temperature and dose. The radiometer measurements were 97.5 $\mu W/cm^2$ and 91.5 $\mu W/cm^2$ at 254 and 185 nm, respectively. Exposure doses estimated by Eq. (1) ranged from 5.82 to 58.2 $mJ/cm^2$.

Liquid absorbance is one of the most important physical characteristics when applying a UV treatment for food pasteurization. As the UV absorbance of a liquid medium increases, the penetration and intensity of UV light delivered to the product decreases, thereby reducing the effectiveness of the delivered UV dose (Koutchma et al., 2009, chap. 1). Fig. 2 shows the UV absorbance spectra (185–300 nm) of the enzymes dissolved in phosphate buffer (pH 7.0) and sodium-acetate buffer (pH 4.0). At 185 nm, absorbance values were 2.03, 2.01 and 1.87 at pH 7.0, and 2.28, 2.27 and 2.30 at pH 4.0 whereas at 254 nm, absorbance values were 0.073, 0.069 and 0.129 at pH 7.0, and 0.079, 0.062 and 0.094 at pH

4.0 for LOX, POD and PPO, respectively. The buffer solutions had different absorbance at different wavelengths with higher absorbance at 185 nm than that at 254 nm. It is known that UV light is strongly absorbed by water at UV range and that fact limits the germicidal effect (Koutchma et al., 2009, chap. 1). Absorbance values at pH 4.0 were slightly higher than those at pH 7.0 at any wavelength due to higher absorbance of acetate buffer.

Table 2 shows results of the effects of UV-C processing factors on residual enzyme activity from the screening experiments. Exposure time (UV-C dose) was found to be the most influential factor on LOX and POD activity whereas temperature was the most influential factor on PPO activity. These factors exhibited negative values indicating that higher levels of those factors produced lower enzyme activity. Wavelength (185 and 254 nm) had no significant effect on LOX and POD activities whereas all factors significantly influenced PPO activity with pH (4.0 and 7.0) being the least influential. The screening design showed a potential synergism between temperature and UV-C treatment to reduce enzyme activity. A more complete experimental design was conducted to better characterize those interactions.

### 3.2. Response surface model for the effects of UV-C processing factors on enzyme activity

A central composite design (CCD) experiment was conducted based on the screening design results as pH, time and temperature factors significantly influenced enzyme activity and thus were selected for a more complete study (Table 3). Wavelength factor was kept at 254 nm. Levels for each factor were selected to mimic food processing conditions (thermal and nonthermal) for common liquid vegetable and fruit matrices (juices and beverages). Coded levels were used to facilitate interpretation of the model coefficients since the codes remove the effect of scale for the three factors.

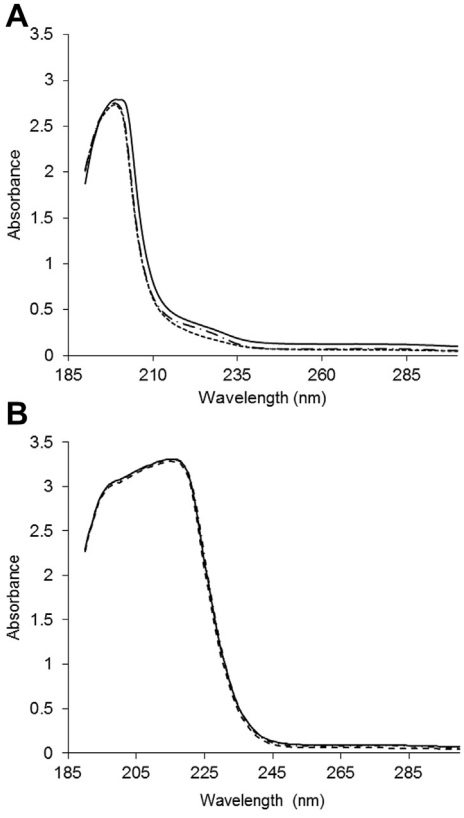


**Fig. 2.** Absorbance values at wavelength of 185–300 nm for LOX (—·—), POD (……) and PPO (——) enzymes in sodium-phosphate buffer at pH 7.0 (A) and sodium-acetate buffer at pH 4.0 (B).

**Table 2**
LOX, POD and PPO enzyme activities as affected by treatment temperature ($T$), treatment time ($t$), pH and wavelength ($W$) of UV-C.

| Factors | | | | $A_{res}$[a] | | |
|---|---|---|---|---|---|---|
| $T$ (°C) | $t$ (min) | pH | $W$ (nm) | LOX | POD | PPO |
| 25 | 1 | 4 | 185 | 0.86 ± 0.02 | 0.71 ± 0.01 | 1.13 ± 0.02 |
| 65 | 1 | 4 | 254 | 0.74 ± 0.05 | 0.64 ± 0.06 | 0.11 ± 0.00 |
| 25 | 10 | 4 | 254 | 0.54 ± 0.05 | 0.15 ± 0.00 | 0.77 ± 0.02 |
| 65 | 10 | 4 | 185 | 0.28 ± 0.03 | 0.004 ± 0.001 | 0.006 ± 0.001 |
| 25 | 1 | 7 | 254 | 0.84 ± 0.02 | 0.94 ± 0.00 | 0.88 ± 0.00 |
| 65 | 1 | 7 | 185 | 0.72 ± 0.03 | 0.72 ± 0.13 | 1.23 ± 0.00 |
| 25 | 10 | 7 | 185 | 0.30 ± 0.05 | 0.31 ± 0.03 | 0.78 ± 0.01 |
| 65 | 10 | 7 | 254 | 0.17 ± 0.02 | 0.01 ± 0.00 | 0.01 ± 0.00 |
| Effect of $T$ | | | | −0.158* | −0.183* | −0.550* |
| Effect of $t$ | | | | −0.467* | −0.636* | −0.446* |
| Effect of pH | | | | −0.095* | 0.120* | 0.220* |
| Effect of $W$ | | | | 0.034 | 0.001 | −0.344* |

*Statistically significant ($p < 0.05$).
[a] Residual activity. Mean ± standard deviation of $n = 3$ values.

Table 4 shows the CCD results of the response of the residual LOX, POD and PPO activities to UV-C processing factors. Average residual activity values for POD, LOX and PPO were 0.532, 0.706 and 0.755 indicating that PPO was the most UV-resistant enzyme. As a general trend, increasing exposure time (UV dose) and temperature in an acidic environment enhanced enzyme inactivation (Figs. 3–5). The results showed UV was able to inactivate the enzymes at low temperatures (25, 35 and 45 °C). For example, UV-C light was able to reduce LOX, POD and PPO activities by 46, 82 and 33% and 70, 69 and 22% after 10 min of UV exposure (UV dose: 58.2 mJ/cm$^2$) at 25 °C at pH 4 and 7, respectively. In order to better comprehend the synergism among temperature and UV-C, enzymes were processed by temperature alone. Treatment at 25 and 35 °C for 10 min at either pH 4 or 7 had no effect on the enzyme activities whereas treatment at 45 and 55 °C had little effect on the activities (4–18% reduction), suggesting the reductions shown in the present study at temperatures below 55 °C were due mostly to UV radiation (data not shown).

Processing conditions of 60 °C and 58.2 mJ/cm$^2$ UV exposure at any pH value produced a complete reduction of enzyme activity. High temperature is known to favor enzyme inactivation by inducing aggregation, dissociation and denaturation effects involving destabilization of covalent as well as non-covalent interactions (Ludikhuyze et al., 2003). The general scheme for thermal enzyme inactivation is often explained by aggregation, dissociation into subunits and denaturation (conformational changes) including a reversible partial unfolding state followed by an irreversible reaction step (Polakovic & Vrabel, 1996; Van Loey et al., 2003). Manzocco, Quarta, and Dri (2009) suggested that the inactivation of PPO by UV-C occurred as a consequence of protein aggregations other than those derived from thermal denaturation. On the other hand, the partial unfolding of enzyme structure by

**Table 3**
Variables and their levels (both uncoded and coded) for the central composite design experiment.

| Variable | Level 1 | Level 2 | Level 3 | Level 4 | Level 5 |
|---|---|---|---|---|---|
| Temperature (°C) | 25 (−2)[a] | 35 (−1) | 45 (0) | 55 (1) | 65 (2) |
| Time (min) | 1.0 (−1.6) | 2.5 (−1) | 5.0 (0) | 7.5 (1) | 10 (2) |
| pH | 4.0 (−2) | 4.75 (−1) | 5.5 (0) | 6.25 (1) | 7.0 (2) |

[a] Coded values are shown in parenthesis (e.g.: −2 = (25 − 45)/10, −1.6 = (1.0 − 5.0)/2.5, +1 = (7.5 − 5.0)/2.5, etc).

**Table 4**
Central composite design for temperature, time and pH on residual enzyme activity of LOX, POD and PPO.

| Parameters | | | | $A_{res}$[a] | | |
|---|---|---|---|---|---|---|
| Run | $T$ (°C) | Time (min) | pH | LOX | POD | PPO |
| 1 | 25 (−2)[b] | 5.0 (0) | 5.50 (0) | 0.83 ± 0.01$^{Ac}$ | 0.67 ± 0.07$^{A}$ | 0.55 ± 0.05$^{A}$ |
| 2 | 55 (+1) | 7.5 (+1) | 4.75 (−1) | 0.42 ± 0.02$^{B}$ | 0.15 ± 0.01$^{B}$ | 0.16 ± 0.02$^{B}$ |
| 3 | 35 (−1) | 2.5 (−1) | 6.25 (+1) | 0.86 ± 0.05$^{A}$ | 0.80 ± 0.06$^{A}$ | 1.20 ± 0.02$^{C}$ |
| 4 | 45 (0) | 5.0 (0) | 5.50 (0) | 0.69 ± 0.02$^{C}$ | 0.56 ± 0.06$^{C}$ | 1.15 ± 0.06$^{C}$ |
| 5 | 55 (+1) | 2.5 (−1) | 6.25 (+1) | 0.88 ± 0.03$^{A}$ | 0.87 ± 0.03$^{D}$ | 1.01 ± 0.14$^{C}$ |
| 6 | 65 (+2) | 5.0 (0) | 5.50 (0) | 0.47 ± 0.11$^{B}$ | 0.28 ± 0.05$^{E}$ | 0.01 ± 0.00$^{D}$ |
| 7 | 55 (+1) | 2.5 (−1) | 4.75 (−1) | 0.67 ± 0.02$^{C}$ | 0.67 ± 0.07$^{A}$ | 0.66 ± 0.05$^{A}$ |
| 8 | 35 (−1) | 2.5 (−1) | 4.75 (−1) | 0.84 ± 0.06$^{A}$ | 0.86 ± 0.03$^{D}$ | 1.16 ± 0.03$^{C}$ |
| 9 | 35 (−1) | 7.5 (+1) | 4.75 (−1) | 0.57 ± 0.02$^{B}$ | 0.35 ± 0.00$^{E}$ | 0.89 ± 0.03$^{E}$ |
| 10 | 55 (+1) | 7.5 (+1) | 6.25 (+1) | 0.73 ± 0.04$^{C}$ | 0.17 ± 0.00$^{B}$ | 0.08 ± 0.02$^{D}$ |
| 11 | 45 (0) | 10.0 (+2) | 5.50 (0) | 0.55 ± 0.02$^{B}$ | 0.19 ± 0.00$^{B}$ | 0.63 ± 0.03$^{A}$ |
| 12 | 45 (0) | 5.0 (0) | 5.50 (0) | 0.71 ± 0.03$^{C}$ | 0.57 ± 0.00$^{C}$ | 1.06 ± 0.04$^{C}$ |
| 13 | 45 (0) | 5.0 (0) | 5.50 (0) | 0.71 ± 0.05$^{C}$ | 0.57 ± 0.04$^{C}$ | 1.02 ± 0.11$^{C}$ |
| 14 | 45 (0) | 5.0 (0) | 7.00 (+2) | 0.83 ± 0.01$^{A}$ | 0.62 ± 0.04$^{C}$ | 0.48 ± 0.06$^{A}$ |
| 15 | 35 (−1) | 7.5 (+1) | 6.25 (+1) | 0.94 ± 0.16$^{A}$ | 0.52 ± 0.07$^{C}$ | 0.90 ± 0.05$^{E}$ |
| 16 | 45 (0) | 5.0 (0) | 5.50 (0) | 0.64 ± 0.06$^{C}$ | 0.53 ± 0.01$^{C}$ | 1.08 ± 0.06$^{C}$ |
| 17 | 45 (0) | 5.0 (0) | 5.50 (0) | 0.78 ± 0.02$^{A}$ | 0.54 ± 0.07$^{C}$ | 1.06 ± 0.07$^{C}$ |
| 18 | 45 (0) | 1.0 (−1.6) | 5.50 (0) | 0.83 ± 0.10$^{A}$ | 0.89 ± 0.07$^{D}$ | 0.75 ± 0.08$^{E}$ |
| 19 | 45 (0) | 5.0 (0) | 5.50 (0) | 0.70 ± 0.02$^{C}$ | 0.64 ± 0.07$^{A}$ | 1.15 ± 0.01$^{C}$ |
| 20 | 45 (0) | 5.0 (0) | 4.00 (−2) | 0.46 ± 0.03$^{B}$ | 0.19 ± 0.03$^{B}$ | 0.10 ± 0.02$^{D}$ |

[a] Residual activity. Mean ± standard deviation of $n = 3$ values.
[b] Coded values are shown in parenthesis.
[c] Different letters indicate statistical difference among treatments ($p < 0.05$).

thermal processing could enhance the UV-C light absorption of conjugated double bonds leading to enzyme inactivation (Guerrero-Beltran & Barbosa-Cánovas, 2006). This could explain the positive interaction observed between temperature and UV-C exposure time. Changing the environmental conditions such as pH may also perturb the enzyme conformation resulting in lower thermal and UV-C stability.

The values of specific residual enzyme activities (Table 4) were fit to a second-order polynomial equation (Eq. (7)) to characterize the effects of UV-C processing factors on LOX, POD and PPO activities (Table 5). Linear terms (temperature, time and pH) had a significant effect on enzyme activity ($p < 0.05$). The most influential factor for LOX was pH ($F$ value 69.2), for POD was exposure time ($F$ value 250.3) and for PPO was temperature ($F$ value 40.0) suggesting LOX was more influenced by the acidic environment, POD was the most UV-C labile enzyme whereas PPO was more thermolabile. The differences in enzyme resistance have been explained by the fact of the presence of isoenzymes with different thermal resistance. This could be the case for LOX and POD where several labile and resistant forms have been observed in different vegetable matrices (Anese & Sovrano, 2006; Morales-Blancas, Chandia, & Cisneros-Zevallos, 2002). However, surprisingly POD was found to be the most UV-C labile when POD is commonly used as an index for blanching due to their high thermal resistance. This could mean UV-C exerts additional inactivation effects when combined with temperature. A significant interaction ($p < 0.05$) was found between time and pH for LOX and temperature and time for POD and PPO, indicating synergistic effects. The regression analysis of the data set showed a high model F value (18.1, 38.9 and 16.7) for LOX, POD and PPO, respectively, suggesting that the polynomial model can be successfully used to predict the experimental data ($p < 0.05$). $R^2$ values for LOX, POD and PPO equations were 0.84, 0.92 and 0.83 and RMSE values 0.07, 0.08 and 0.18 indicating prediction errors of 7, 8 and 18%, respectively.

Fig. 3 shows the contour plots of the predicted residual LOX activity over independent variables (pH and time) at different temperatures (25, 45 and 65 °C). The model shows that LOX enzyme was UV-resistant and less effect was noticed at basic pH conditions (pH 6–7). However, acidic environments (pH 4–5) seemed to exert a stronger effect and the model indicates a linear decrease of enzyme activity with decreasing pH value with complete inactivation at pH 4.5 after 58.2 mJ/cm$^2$ (10 min) UV-C exposure at 65 °C. The model showed that the linear decrease of enzyme activity was more pronounced at higher temperatures. However, at 25 °C and 45 °C, predicted reduction reached only about 50% and 85%, respectively, after 58.2 mJ/cm$^2$ UV exposure even at pH of 4.0. Some authors have studied the effects of different UV-C doses (2.5–5.0 J/cm$^2$) on LOX activity as it relates to fruit ripening and senescence (Barka, 2001; Gonzalez-Aguilar et al., 2007). The studies showed an increase in LOX activity in tomato (up to 3 d with a decrease during 30 d) and mango (3 and 4-fold increase after 24 h remained stable during storage of 18 d) fruits after UV-C treatment. Our present study demonstrated that LOX is the most resistant enzyme to UV-C processing in the model system.

Fig. 4 shows the contour plots of the predicted residual POD activity over independent variables (temperature and time) at different pH values (7.0, 5.5 and 4.0). POD was a UV-labile enzyme and increasing the exposure time produced a linear decrease of enzyme activity at any temperature value. The polynomial equation indicates that a decrease in pH value produced a larger zone of complete enzyme reduction indicating a higher sensitivity of POD to UV-C processing. The model also predicts that, at pH 7.0, conditions for complete inactivation were narrowed to the range of 55–65 °C and 8–10 min whereas at pH 4.0 conditions were expanded to 5–10 min and 40–65 °C. Chisari et al. (2011) studied the effects of UV-C irradiation on POD activity in fresh-cut melon. UV-C treatment (30–120 s, 40 J/s/m$^2$) had the same effectiveness as 100 ppm NaOCl washing (18% inactivation) but enzyme activity increased during storage for 10 d at 5 °C indicating *de nova* synthesis of the enzyme by the fruit. Noci et al. (2008) showed negligible effects of UV-C batch processing after 30 min exposure time on POD activity in fresh apple juice (pH 3.71) due possibly to the high liquid absorbance of the matrix. Another study conducted by Falguera et al. (2011) showed that UV treatment reduced POD activity to undetectable level in apple juice made from four different apple varieties after 15 min of UV exposure time (light intensity, $3.88 \times 10^{-7}$ E/s) in comparison with 40 and 100 min needed for a complete inactivation of PME and PPO, respectively. That study

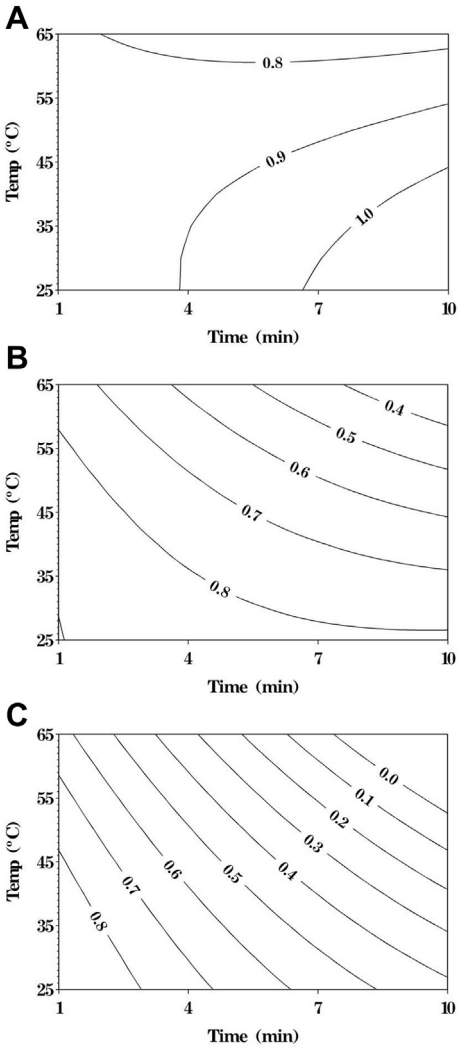


**Fig. 3.** Contour plots showing predicted residual LOX activity as a function of temperature and time at pH 7.0 (A), pH 5.5 (B) and pH 4.0 (C). UV-C was applied at wavelength of 254 nm. Numbers inside the contour plots indicate residual enzyme activity.

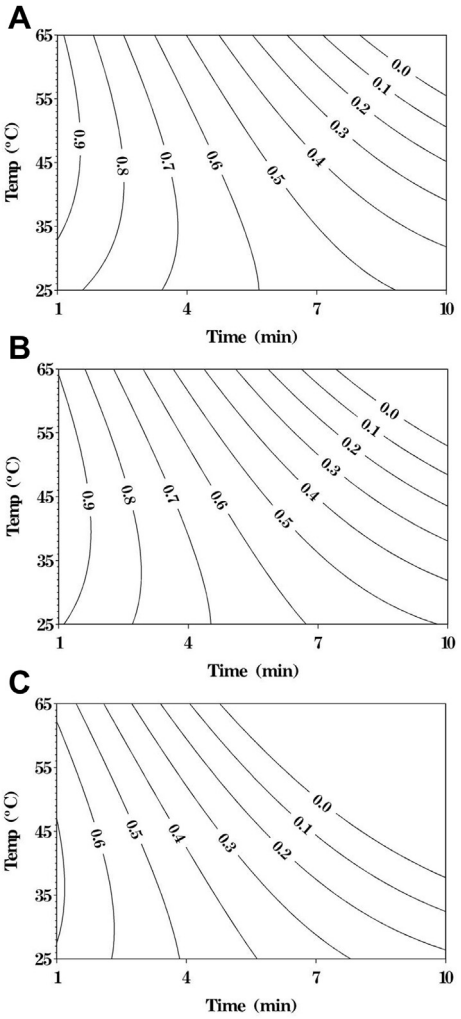


**Fig. 4.** Contour plots showing predicted residual POD activity as a function of temperature and time at pH 7.0 (A), pH 5.5 (B) and pH 4.0 (C). UV-C was applied at wavelength of 254 nm. Numbers inside the contour plots indicate residual enzyme activity.

shows POD as the most UV-labile enzyme which is in agreement with our findings. However, the UV exposure times for a complete enzyme reduction (10 min) are shorter due possibly to the synergetic effects of the combination of UV-C with a thermal treatment or different dose intensity. In a recent study, Neves, Vieira, and Silva (2012) also found a synergistic effect of UV-C and blanching temperature above 85 °C on POD inactivation in zucchini.

Fig. 5 shows the contour plots of the predicted residual PPO activity over independent variables (temperature and time) at different pH values (7.0, 5.5 and 4.0). The model predicted that PPO was the most thermolabile enzyme and indicated that temperatures higher than 55 °C and longer than 2, 5 and 1 min of exposure times at pH 7.0, pH 5.5 and pH 4.0, respectively, led to a complete enzyme inactivation. The enzyme was found to be more resistant around 35 °C after 1–5 min at pH 5.5 and departure from those conditions produced higher inactivation. Guerrero-Beltran and Barbosa-Cánovas (2006) applied a UV-C treatment to mango nectar (pH 3.8) at room temperature and measured the remaining PPO activity. The authors estimated *D* values (90% of enzyme inactivation) for PPO of 152–199 min (2280–2991 J/cm$^2$). Energy values estimated in our study as 0.31–2.82 kJ using water as reference (calculating the energy used to heat the sample up to 65 °C) were found to be much lower than energy applied in other studies. The synergistic combination of temperature and UV-C exposure time applied in this study led to a complete PPO inactivation (99%) by saving energy applied to the process.

In other studies (Chisari et al., 2011; Gonzalez-Aguilar et al., 2007), UV-C was applied as a post-harvest method to delay the ripening and senescence of whole or cut fruit. It has been shown that enzyme activities increased following UV treatment (Barka, 2001; Gonzalez-Aguilar et al., 2007). The experimental data obtained in the liquid model system in the present study was much more relevant to the use of UV as a preservation method for liquid foods. Studies have shown almost complete inactivation of PPO in mango nectar using UV and significant reductions of PPO and POD activities in apple juice when high exposure times and energy are applied (Falguera et al., 2011; Guerrero-Beltran & Barbosa-Cánovas, 2006; Noci et al., 2008). However, few earlier studies (if any) have been conducted to use a combination of mild temperatures (25–65 °C) and UV radiation to enhance the inactivation of enzymes. In our present study, the effects of UV dose, temperature, and pH levels were all taken into account using the central composite design and polynomial models. Our present study demonstrated

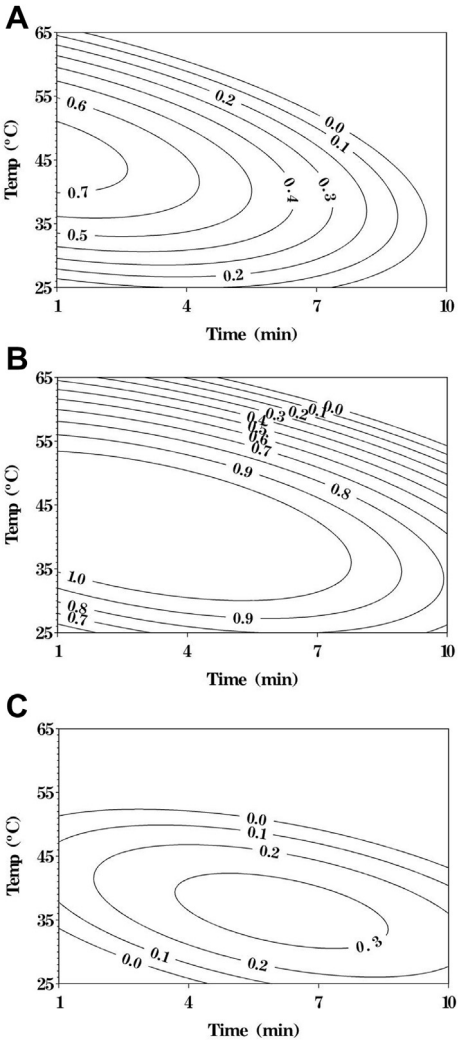


**Fig. 5.** Contour plots showing predicted residual PPO activity as a function of temperature and time at pH 7.0 (A), pH 5.5 (B) and pH 4.0 (C). UV-C was applied at wavelength of 254 nm. Numbers inside the contour plots indicate residual enzyme activity.

that, similar to other nonthermal processing technologies such as high pressure (Ludikhuyze et al., 2003) and pulsed electric fields (Zhao, Yang, & Zhang, 2012) where temperature is combined with pressure or electric field to obtain enzyme inactivation, UV-C needs to be combined with temperatures higher than 60 °C in order to inactivate the enzymes (more than 95%, below the detection limit).

**Table 5**
Estimated parameters for coded levels and fit statistics for LOX, POD and PPO enzymes using the response surface regression model.

| Parameters | LOX | POD | PPO |
|---|---|---|---|
| Intercept | 1.142 | −1.346 | −12.627 |
| *T* | −0.008* | 0.009* | 0.143* |
| *t* | −0.167* | −0.040* | 0.402* |
| pH | 0.020* | 0.715* | 3.580* |
| *T***T* | $-0.008 \times 10^{-3}$ | −0.0001 | −0.001 |
| *T***t* | −0.001 | −0.002* | −0.004* |
| *T**pH | 0.002 | 0.001 | 0.003 |
| *t***t* | 0.001 | 0.002 | −0.010 |
| *t**pH | 0.030* | 0.004 | −0.029 |
| pH*pH | −0.013 | −0.065 | −0.318 |
| *F* value | 18.1 | 38.9 | 16.7 |
| $R^2$ | 0.844 | 0.921 | 0.833 |
| RMSE | 0.068 | 0.077 | 0.185 |

*Statistically significant ($p < 0.05$).

## 4. Conclusions

The combination of temperature and UV-C exposure (60 °C-58.2 mJ/cm$^2$) resulted in a complete enzyme reduction at any pH value while using less energy in the process (lower temperature and/or shorter UV-C exposure time). The treatment efficacy was increased under an acidic environment. Wavelength factor seemed to have a negligible effect on enzyme activity. Second-order polynomial equations explained the effects of processing and environmental factors on enzyme activity with a low fitting error. PPO was found to be the most UV-resistant enzyme whereas POD was the most UV-labile. UV doses applied in this study were of orders of magnitude lower than those applied by other authors in different vegetable matrices. These experimental data will serve as the basis for further development of UV-C processes in liquid matrices.

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## References

Aguiló-Aguayo, I., Sobrino-López, A., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Influence of high-intensity pulsed electric field processing on lipoxygenase and β-glucosidase activities in strawberry juice. *Innovative Food Science & Emerging Technologies, 9*, 455–462.

Anese, M., & Sovrano, S. (2006). Kinetics of thermal inactivation of tomato lipoxygenase. *Food Chemistry, 95*, 131–137.

Barka, E. (2001). Protective enzymes against reactive oxygen species during ripening of tomato (*Lycopersicon esculentum*) fruits in response to low amounts of UV-C. *Australian Journal of Plant Physiology, 28*(8), 785–791.

Basak, S., & Ramaswamy, H. S. (1996). Ultra high pressure treatment of orange juice: a kinetic study on inactivation of pectin methyl esterase. *Food Research International, 29*(1), 601–607.

Cano, M., Hernandez, A., & De Ancos, B. (1997). High pressure and temperature effects on enzyme inactivation in strawberry and orange products. *Journal of Food Science, 62*(1), 85–88.

Chisari, M., Barbagallo, R. N., Spagna, G., & Artes, F. (2011). Improving the quality of fresh-cut melon through inactivation of degradative oxidase and pectinase enzymatic activities by UV-C treatment. *International Journal of Food Science and Technology, 46*(3), 463–468.

Falguera, V., Pagan, J., & Ibarz, A. (2011). Effect of UV irradiation on enzymatic activities and physicochemical properties of apple juices from different varieties. *LWT – Food Science and Technology, 44*(1), 115–119.

FDA, Food and Drug Administration. (2012). *Title 21 – Food and drugs, chapter I – Food and Drug Administration Department of Health and Human Services, part 179 – Irradiation in the production, processing and handling of food, subpart B-radiation and radiation sources, sec. 179.39 ultraviolet radiation for the processing and treatment of food*. Washington, DC: U.S. Government Printing Office.

Garcia-Palazon, A., Suthanthangjai, W., Kajda, P., & Zabetakis, I. (2004). The effects of high hydrostatic pressure on beta-glucosidase, peroxidase and polyphenoloxidase in red raspberry (*Rubus idaeus*) and strawberry (*fragaria × ananassa*). *Food Chemistry, 88*(1), 7–10.

Gonzalez-Aguilar, G. A., Zavaleta-Gatica, R., & Tiznado-Hernandez, M. E. (2007). Improving postharvest quality of mango 'haden' by UV-C treatment. *Postharvest Biology and Technology, 45*(1), 108–116.

Guerrero-Beltran, J., & Barbosa-Cánovas, G. (2004). Review: advantages and limitations on processing foods by UV light. *Food Science and Technology International, 10*(3), 137–147.

Guerrero-Beltran, J., & Barbosa-Cánovas, G. (2006). Inactivation of *Saccharomyces cerevisiae* and polyphenoloxidase in mango nectar treated with UV light. *Journal of Food Protection, 69*(2), 362–368.

Koutchma, T. (2009). Advances in ultraviolet light technology for non-thermal processing of liquid foods. *Food and Bioprocess Technology, 2*(2), 138–155.

Koutchma, T., Forney, L., & Moraru, C. (2009). *Ultraviolet light in food technology: Principles and applications*. Boca Raton: CRC.

Ludikhuyze, L., Van Loey, A., Indrawati, S. C., & Hendrickx, M. (2002). High pressure processing of fruit and vegetables. In W. M. F. Jongen (Ed.), *Fruit and vegetable processing: Improving quality* (pp. 346–362). Cambridge: Woodhead Publishing.

Ludikhuyze, L., Van Loey, A., Indrawati, S. C., & Hendrickx, M. (2003). Effects of combined pressure and temperature on enzymes related to quality of fruits and vegetables: from kinetic information to process engineering aspects RID C-9342-2009. *Critical Reviews in Food Science and Nutrition, 43*(5), 527–586.

Manzocco, L., Quarta, B., & Dri, A. (2009). Polyphenoloxidase inactivation by light exposure in model systems and apple derivatives. *Innovative Food Science and Emerging Technologies, 10*, 506–511.

Morales-Blancas, E. F., Chandia, V. E., & Cisneros-Zevallos, L. (2002). Thermal inactivation kinetics of peroxidase and lipoxygenase from broccoli, green asparagus and carrots. *Journal of Food Science, 67*, 146–154.

Neves, F. I. G., Vieira, M. C., & Silva, C. L. M. (2012). Inactivation kinetics of peroxidase in zucchini (*Cucurbita pepo* L.) by heat and UV-C radiation. *Innovative Food Science & Emerging Technologies, 13*(0), 158–162.

Noci, F., Riener, J., Walkling-Ribeiro, M., Cronin, D. A., Morgan, D. J., & Lyng, J. G. (2008). Ultraviolet irradiation and pulsed electric fields (PEF) in a hurdle strategy for the preservation of fresh apple juice. *Journal of Food Engineering, 85*(1), 141–146.

Polakovic, M., & Vrabel, P. (1996). Analysis of the mechanism and kinetics of thermal inactivation of enzymes: critical assessment of isothermal inactivation experiments. *Process Biochemistry, 31*(8), 787–800.

Rodrigo, D., Jolie, R., Van Loey, A., & Hendrickx, M. (2007). Thermal and high pressure stability of tomato lipoxygenase and hydroperoxidelyase. *Journal of Food Engineering, 79*, 423–429.

Sampedro, F., Fan, X., & Rodrigo, D. (2010). High hydrostatic pressure processing of fruit juices and smoothies: research and commercial application. In C. J. Doona, K. Kustin, & F. E. Feeherry (Eds.), *Case studies in novel food processing technologies: Innovations in processing, packaging, and predictive modelling* (pp. 34–72). Cambridge: Woodhead Publishing.

Stevens, C., Khan, V. A., Lu, J. Y., Wilson, C. L., Chalutz, E., Droby, S., et al. (1999). Induced resistance of sweet potato to *Fusarium* root rot by UV-C hormesis. *Crop Protection, 18*, 463–470.

Van Loey, A., Indrawati, S. C., & Hendrickx, M. (2003). Inactivation of enzymes from experimental design to kinetic modeling. In J. R. Whitaker, A. G. J. Voragen, & D. W. S. Wong (Eds.), *Handbook of food enzymology* (pp. 49–58). New York: Marcel Dekker.

Weemaes, C. A., De Cordt, S. V., Ludikhuyze, L. R., Van den Broeck, I., Hendrickx, M. E., & Tobback, P. P. (1997). Influence of pH, benzoic acid, EDTA, and glutathione on the pressure and/or temperature inactivation kinetics of mushroom polyphenoloxidase. *Biotechnology Program, 13*, 25–32.

Zhao, W., Yang, R., & Zhang, H. Q. (2012). Recent advances in the action of pulsed electric fields on enzymes and food component proteins. *Trends in Food Science & Technology, 27*, 83–96.